U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 158.

B. T. GALLOWAY, *Chief of Bureau.*

THE ROOT-ROT OF TOBACCO CAUSED BY THIELAVIA BASICOLA.

BY

W. W. GILBERT,

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Issued October 7, 1909.

WASHINGTON:

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., June 5, 1909.

Sir: I have the honor to transmit herewith and recommend for publication as Bulletin No. 158 of the series of this Bureau the accompanying paper, entitled "The Root-Rot of Tobacco Caused by Thielavia Basicola," by Mr. W. W. Gilbert. This paper has been submitted with a view to publication by Mr. W. A. Orton, Pathologist in Charge of Cotton and Truck Disease Investigations.

Within the last few years this disease has become increasingly injurious to tobacco, particularly in the Connecticut Valley, where tobacco is the principal money crop. A study of the fungus causing the disease has been made, and the methods of seed-bed sterilization herein described provide a means of controlling it in the seed beds.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE ROOT-ROT OF TOBACCO CAUSED BY THIELAVIA BASICOLA.

INTRODUCTION.

During the last few years a root-rot of tobacco has caused considerable loss to growers in certain sections of the United States, and the disease has become so prevalent that it must be reckoned with and some means of checking its ravages adopted in order to cultivate the crop successfully. Fortunately the area over which the disease is severe, so far as our present knowledge goes, is not very large, being confined to Connecticut, but the loss in individual cases is nevertheless great. Many instances are known where the entire crop of seedlings raised by a farmer had to be discarded because of the root-rot and healthy plants purchased to take their place, thus entailing not only the loss of his total outlay of work and fertilizers, but in addition the extra expense of buying healthy plants.

In some communities where the trouble has been general there has been considerable difficulty in securing healthy plants, with the result that the excessive demand caused an advance in price, making the actual loss much greater. Before the effects of the disease were more generally understood, additional loss was caused by the setting of diseased plants in the fields. Even though the conditions were the very best for the plants, some were not able to get a foothold and had to be reset, while in many cases the entire crop, after remaining two or three weeks at a standstill with occasional plants dying, has been plowed up and the land reset with plants from other sources. These operations involve a great deal of labor that is absolutely wasted, to say nothing of the time lost to the crop. No small amount of loss has also been occasioned by the fact that healthy plants, when set, have been attacked and the crop so stunted that 75 to 100 per cent has been rendered unmarketable.

DESCRIPTION OF ROOT-ROT.

The root-rot of tobacco is characterized by the blackening and decay of the taproot and lateral roots of seedlings and of larger plants in the field, caused by the growth in their tissues of the fungus

Thielavia basicola Zopf. The macroscopic effects of the disease upon the plants attacked vary considerably with the conditions under which they are grown, but an unhealthy appearance of the leaves is almost always to be noted.

THE DISEASE IN THE SEED BED.

Damping-off.—If attacked while very young, the seedlings are sometimes killed when not more than one-third to one-half inch high, in much the same manner as by "damping-off" fungi except that the fungous hyphæ penetrate not only the portion of the plantlet near the soil line, but also the rootlets and the stem above, spores having been seen protruding from the stem of a seedling all the way from the soil to the cotyledons, as well as on the rootlets.

Effect on roots.—In other cases, when conditions do not favor as severe an attack, the roots alone are directly invaded by the fungus and the plants are stunted. If one of the slightly diseased plantlets is very carefully taken up and the adhering soil washed away, it will be noted upon examination that the tips of a number of the rootlets present a brown or black appearance, according to the length of time the fungus has been working. The browning of the tissues is caused by the disintegration of the root by the fungus living in it parasitically and the black color by the subsequent production of spores. The disease progresses up the roots, both taproot and laterals being attacked, until the entire root system is destroyed, and if such a plant is pulled all the roots break off and remain in the soil, the blackened base of the stem alone showing where the roots were attached. The end of the taproot is most commonly attacked first and then the laterals. (Pl. III, fig. 3, *B*.)

As the root system thus becomes reduced, the plantlet usually puts out numerous lateral roots to take the place of those rendered useless by the disease. These serve to nourish the plantlet for a time, but eventually become diseased and decay.

Effect on leaves.—The appearance of the leaves of diseased seedlings varies with the soil and with the plant food available. When the beds are made of rich soil, heavily fertilized with quickly available materials, such as hen manure or sodium nitrate, the plants may present a very vigorous appearance, though the leaves are usually of a darker green than is customary with perfectly healthy plants. However, such plants exhibit root systems very badly diseased, not infrequently the taproot breaking off just below the surface of the soil; this has been noted even with plants 4 to 6 inches tall.

In other cases, where the soil is poor and little quickly available fertilizer has been used, the plantlets are distinctly stunted and the leaves have a sickly yellow color, which appears first at the tips, but

gradually pervades the whole leaf. Few, if any, new feeding roots are produced, and the plant makes little appreciable growth. The beds present a very uneven appearance, the plants being large in some spots and in others small and scattered.

In any case the result is the same; the plants are rendered unfit for setting and the beds are a total loss, the farmer being obliged to purchase healthy plants when his own beds should have produced more than enough plants to set his fields.

THE DISEASE IN THE FIELD.

When badly diseased plants are set in the field they generally remain for two or three weeks without any apparent growth, some yellowing, wilting, and dying during that period. Finally a large portion of a field set with diseased plants has to be reset. If the soil is of a light, open texture the survivors may eventually gain the ascendency over the disease and under favorable circumstances produce a fairly good, though late, crop, but in the heavier soils the disease persists, and the tobacco, though making some growth, is permanently crippled and produces few, if any, marketable plants. Cases have been observed where diseased plants have reached maturity at a height of 8 to 15 inches, the root systems being a mass of decayed rootlets, all breaking off to within a few inches of the base of the stem when the plants were pulled. (Pl. III, fig. 2.)

In the spring of 1906 three cases came to the attention of the writer in which fields of from 1 to 4 or 5 acres were set with diseased plants, and after ten days or two weeks plowed up and reset, because the plants were so badly affected that they made no growth whatever and gave no promise of it. In one case, in a field of several acres, the majority of the plants were so badly diseased and stunted that the crop was not worth harvesting. More generally, however, the trouble is worse in spots over the field. (Pl. V, fig. 1.) It matters little whether diseased plants are set in a field where no infection is present or whether healthy plants are set in a field already diseased; the result is the same, and where the conditions are favorable for the development of the disease the crop is seriously injured.

When large roots are attacked by the fungus, the tissues, being older and tougher, are not as easily penetrated as those of smaller roots, and the growth, except in very severe cases, is generally confined to the outer surface, where a brownish, scurfy appearance is to be noted, and sometimes an enlargement of the root at the point attacked.

Where whole fields become infected in the manner described, the problem becomes complicated, since methods that might be practical in the treatment of restricted areas used for seed beds would be far too costly for use in large fields.

DESCRIPTION OF THE FUNGUS CAUSING ROOT-ROT.

Thielavia basicola, the fungus which causes the root-rot of tobacco, belongs to the ascomycetous family Perisporiaceæ. It is characterized by the production of three kinds of spores, known, respectively, in the order of their appearance, as endoconidia, chlamydospores, and ascospores.

MYCELIUM.

The mycelium consists of much branched hyphæ, generally much septate, but sometimes sparingly so, hyaline at first, but later becoming slightly brown. The fungous threads are very small, the diameter of the cells varying in culture from 3 to 7 microns. Moreover, some hyphæ are strictly linear, while others are very irregular in outline. When young the cells of the mycelium are filled with granular protoplasm, but in old cultures the protoplasm is less abundant.

The manner of branching is quite characteristic in that it is in many cases almost dichotomous, the branch being thrown out from the apex of a cell of the main hypha, where a slight enlargement takes place and a cell wall develops, cutting off the branch from the main hypha.

ENDOCONIDIOPHORES.

Arising from the mycelium at various points, sometimes singly, sometimes in clusters, are hyaline to dingy brown thin-walled branches, which form endoconidiophores of a few to several short, plump, barrel-shaped cells, terminated by a long tapering cell within which the endoconidia are produced. Zopf has given these endoconidiophores the characteristic name "pistolenförmige Conidien-bildungen," pistol-shaped conidial formations. (Pl. I, figs. 4 and 5.)

ENDOCONIDIAL CELL.

The terminal or endoconidial cell is slightly swollen at the base and gradually tapers to the long, almost linear delivery tube through which the conidia are discharged. In length the cells vary from 90 to 170 μ, with a basal diameter of 5 to 6 μ, and they are 3 to 5 μ in diameter at the mouth of the delivery tube. (Pl. I, fig. 4.)

The endoconidia are produced from the copious protoplasm within the terminal cell, which opens by a bursting or dissolution of the tip, and the conidia are slowly pushed out by the growth of the protoplasm in the swollen basal portion of the cell, new conidia being formed continuously in the rear of those being ejected. It is sometimes difficult to perceive that the conidia originate within the terminal cell and are not formed by its direct septation, as the walls of the

conidia and of the delivery tube are so thin and transparent and fit so closely together that a high power is necessary to discern this point. Quite frequently, however, a conidium will be found projecting part way out of the delivery tube, or, again, in old cultures the last conidium may have been knocked out, leaving the end of the delivery tube empty, so that the fringed or notched orifice is visible. In young cultures the conidia are produced in great numbers, giving a silvery white appearance, the conidia being pushed out in long lines or, in case the conidiophore is erect, falling in little piles. Aderhold has counted 160 conidia which came from a single endoconidial cell, and the writer has seen 40 in drop cultures twenty-four hours old.

ENDOCONIDIA.

When young the endoconidia are hyaline, thin walled, linear, and very slightly rounded at the ends. When about to germinate they swell and assume an ovoid shape. The contents of the conidia are very transparent, with a large vacuole in each end. In old cultures conidia have been observed of a slightly brownish color and with thickened cell walls. Conidia vary from 8 to 23 μ in length by 3 to 5 μ in diameter, the average being about 15 by 4 μ. This fructification is the most characteristic of Thielavia, since such a conidial formation is of very infrequent occurrence. (Pl. I, fig. 1.)

In corn-meal cultures conidia are produced in profusion in two to three days, and they doubtless serve as the most prolific means of dissemination of the disease, as they may germinate at once, while the chlamydospores and ascospores produced later require at least a short period of rest before being able to start new growth.

CHLAMYDOSPORES.

The chlamydospores, second in order of production, are often borne on the same hypha as the endoconidiophores, on short lateral branches put out from just below them; or they may be produced from any portion of the mycelium. They are sometimes borne singly, but more often in many-branching clusters. These spores are much thicker walled than the conidia and are more richly supplied with protoplasm and reserve food material in the form of oil globules, and thus are adapted to serve as resting spores. In color they vary from a brownish black to an olive-brown, in mass usually giving a dull black color. Most writers have considered them as single spores borne in chains of three to six, but they are more properly treated as compound spores made up of two to three basal cells surmounted by one to eight fertile segments. The basal cells are thin walled, hyaline, and sterile, while the fertile cells are dark brown

and surrounded by thick walls, and eventually separate into short cylindrical individual cells, each one of which is capable of germination.

The compound spores are club shaped, usually tapering slightly from the rounded terminal cell to the first sterile basal cell, and then more abruptly to the hypha, on which they are borne. In length the chlamydospores vary from 25 to 65 μ, the majority being about 45 μ, with a diameter of 10 to 12 μ, the individual segment measuring from 5 to 18 μ in length. There are slight constrictions at the points of division between the segments, the cross walls being almost invariably perpendicular to the long axis of the compound spore. The exterior of the spores is slightly roughened. Many forms of branching are found. Sometimes a main hypha bears spores singly on all sides at close intervals; in other cases they are borne in close, broom-like clusters, several spores emanating from one place, or one spore from a sterile segment at the base of another, a third from the base of this, and so on until a dense bunch of spores results. In some instances one, two, or three spores have been observed to originate from another compound spore. (Pl. I, figs. 5 and 6.)

ASCOSPORES.

The ascosporic stage, the last to develop, is found abundantly on old diseased roots of tobacco, but the writer has never succeeded in getting it in pure cultures. Peglion (37) [a] alone, of all who have studied the fungus in culture, reports having obtained it on old potato cultures to which was added sterilized water acidulated with 0.6 per cent of tartaric acid and the tubes kept in the thermostat at 25° C. for a week.

The perithecia are borne in the midst of the other spore forms. The writer was fortunate enough to observe a perithecium attached to the same hypha which bore an endoconidiophore. The perithecia are round, dark brown, rough, and thick walled, and without any aperture for the escape of the ascospores. (Pl. I, fig. 8.) In diameter the perithecia vary from 80 to 100 μ, and within them are produced a number of fragile, egg-shaped asci, in each of which are developed eight oblong, nonseptate, slightly spindle-shaped spores, of a chocolate-brown color when ripe, thick walled, and provided with a large central oil drop. (Pl. I, fig. 9.) The spores measure 8 to 10 μ by 4 to 5 μ and are set free within the perithecium by the deliquescence of the asci walls before the spores are ripe. It is therefore exceedingly difficult to observe the asci. The ascospores are released by the disintegration

[a] The numbers in parentheses refer to the bibliography at the end of the bulletin.

or rupture of the perithecium, which takes place soon after the ripening of the spores; consequently, spores are rarely found in the perithecium on old diseased roots, but free. These spores have never been observed to germinate by the writer. Although hundreds of examinations of both fresh material and microtome sections cut for the purpose have been made by the writer, thus far no asci have been found. They are, however, figured by Zopf (58), and the writer has no reason to question their existence.

MEANS OF DISSEMINATION.

From the observations of Sorauer (48) it would seem probable that Thielavia is rather widely distributed in soils of abundant humus content, and particularly in leaf mold. This is borne out by the fact of its known occurrence in at least seven countries of the world. Its introduction into tobacco seed beds may occur in a number of ways. Leaf mold is sometimes used in the preparation of the beds, and the fungus may thus be brought in. Infected soil clinging to the feet of men or animals or to tools and the custom of buying seedlings for setting which may have the disease so slightly as not to be noticed are probably common agencies in the transmission of the trouble from one seed bed to another. Instances have come to the writer's notice where a single spot in a seed bed showed the disease one year, but by the next the entire bed and others adjacent had become infected, the spores having been scattered when the beds were prepared and through watering and subsequent cultivation and weeding.

PARASITISM OF THE FUNGUS.

Investigators of Thielavia almost without exception have acknowledged its parasitic nature to a degree, some, however, believing it to be a very weak parasite very largely dependent on conditions, and a few being in doubt as to its true nature.

CONCLUSIONS OF VARIOUS WRITERS.

It was Berkeley's (5) opinion that the fungus " was either destructive of the plant on which it grew or was developed on it in consequence of previous disease."

Zopf (59) characterized it as an undoubted parasite attacking the root systems of a number of plants, causing serious injury, and in some cases even their death.

Sorauer (48) concluded from his study of Thielavia causing the root-brown of cyclamens, that, though it was quite generally to be found living saprophitically in soil rich in humus, it could not always attack plants, but only when they were especially susceptible from

some other reason, such as excessive manuring, too abundant watering, or too high temperature.

Peglion (33), while recognizing the parasitic as well as the saprophytic mode of life of the fungus, stated that the extensive injuries to young tobacco plants might probably be attributed in part to improper cultural conditions, especially excessive watering, the presence of too much humus, and the too close proximity of the roots to the manure used for making the hotbeds. The tender rootlets being thereby injured were readily attacked by the fungus.

Selby (43), Benincasa (3), and Campbell (11) all considered the fungus an active parasite of tobacco, while Aderhold (1) concluded from his inoculation experiments with several plants not reckoned among its hosts that it was not vigorously parasitic and caused much injury only under certain conditions. Galloway (25) likewise held that it was only a weak parasite on violet stems and roots, and that loss could be avoided by the exercise of special care in the selection of soil and in transplanting.

Clinton (19), though fully satisfied as to the parasitism of Thielavia in the seed beds, was doubtful whether or not "the very conspicuous trouble in certain of the tobacco fields of Suffield, Conn., was primarily and chiefly due to the attacks of the fungus."

After a detailed study of the fungus in field, laboratory, and greenhouse extending over three years, the writer has no doubt of the parasitism of Thielavia on the tobacco plant in seed bed and field, though the virulence of its attacks, like those of other fungi on their hosts, varies considerably with the conditions of its environment.

DEMONSTRATION OF PARASITISM OF THIELAVIA.

Petri-dish experiments.—To demonstrate the parasitism of Thielavia, the following experiments were carried out: Four large petri dishes were fitted with double layers of white blotting paper moistened with Koch's nutritive solution and sterilized in the autoclave. All were then sown to tobacco, the seed being scattered rather thickly over the surface of the upper layer of blotting paper and the dishes placed in the thermostat at 30° C. for one and one-half days and then transferred to room temperature and placed in the light. After four days, when the seeds were beginning to germinate, two dishes were watered with sterile water which had been thoroughly inoculated with Thielavia, the remaining dishes receiving the same amount of sterile water. A few days later the seedlings in the inoculated plates were beginning to go down in a manner similar to that of plants attacked by damping-off fungi, and at the end of two weeks every plant had been killed. Examination of the plants attacked showed the presence of Thielavia in every instance. The check dishes showed no

traces of Thielavia, and the plants remained perfectly healthy until the termination of the experiment. This experiment was repeated several times, and almost identical results were secured in every instance. (Pl. II.)

Pot experiments.—To further demonstrate the parasitism of the fungus under more natural conditions, on November 26, 1906, six 8-inch greenhouse pots were filled with rich soil from a badly diseased Connecticut seed bed and autoclaved one and one-half hours at 117° C. On November 28 all were sown to Connecticut Havana tobacco, and on November 30 one pot was inoculated with Thielavia by mixing a considerable quantity of spores from a pure culture with a beaker of sterile water and watering the pot therewith. On January 2, 1907, a second pot was inoculated in the same manner. On January 12 a number of plants were found damped-off in several of the pots, both inoculated and uninoculated. Upon examination Thielavia was found in the dead plants from inoculated pots, but the trouble in the uninoculated pots was due to some other cause, no Thielavia being present.

On April 4 the experiment was terminated, the plants in all six pots being examined, with the following results:

The plants remaining in the four check pots, uninoculated, were found perfectly healthy, roots clean and white.

The plants in the pot inoculated November 30 were very badly diseased, the majority of the roots being blackened and rotted in some degree; Thielavia abundant.

The plants in the pot inoculated January 2, 1907, were even more severely diseased, the roots of every one being blackened and dead to the stem and in some cases up the stem one-eighth to one-half inch.

The experiment was repeated several times under slightly different conditions with virtually the same results in all cases. (Pl. IV, fig. 3.)

HISTORICAL DATA ON ROOT-ROT.

DISCOVERY OF THIELAVIA ON TOBACCO IN THE UNITED STATES.

It is not definitely known just how long the root-rot has been prevalent in tobacco sections of the United States, but data from farmers warrant the belief that it has been causing loss for at least eight or ten years. The amount of loss has been greater within the last three or four years than ever before because of both the wider distribution and the greater virulence of the disease.

Prof. A. D. Selby (43), of the Ohio Agricultural Experiment Station, was the first to report this disease of tobacco in the United States, specimens of White Burley seedlings affected by the trouble having been sent to him from Neville, Clermont County, Ohio, in the

spring of 1899, and the trouble reported again from Germantown in 1903. In Connecticut the root-rot was first brought to public attention by Mr. A. D. Shamel (45), of the Bureau of Plant Industry, in the spring of 1906, a note calling attention to the serious injury caused in the seed beds of the Connecticut Valley being printed in the Hartford Courant for May 28 of that year. Specimens of the diseased plants were sent to the mycologist of the Bureau of Plant Industry, Mrs. F. W. Patterson, and the fungus *Thielavia basicola* causing the trouble was identified by her. About June 1 of the same year the writer was delegated by the Chief of the Bureau of Plant Industry to make a complete study of the trouble with a view to the determination and introduction of some means of reducing or eliminating the loss occasioned. The trouble has since that date been personally located and studied in the seed beds or the fields of 24 tobacco farmers in the Connecticut Valley, with reports of occurrence from numerous other points. and has also been found at one place in Kentucky.

DISCOVERY OF THIELAVIA ON OTHER HOSTS THAN TOBACCO IN THE UNITED STATES.

Thielavia was first reported as a parasite in America by Thaxter (51) in 1891, who found it affecting the roots of violets. Galloway (25) in 1899 studied the trouble on violets and was able to avoid serious loss from its attacks by paying special attention to cultural conditions. About the same date Dr. Erwin F. Smith (46) reports having noted the occurrence of Thielavia in some of his greenhouse experiments, where it attacked cotton and cowpea seedlings, causing rotting of the stems at or beneath the surface of the soil

DISCOVERY OF THIELAVIA ON TOBACCO IN EUROPE.

In Europe root-rot of tobacco was brought to the attention of scientists earlier than in America, as Peglion (33) first reported it in 1897 as prevailing in the seed beds of the tobacco agency at San Sepolcro in north central Italy and stated that the tobacco plant had not up to that time been reckoned among the host plants of Thielavia. Subsequently the trouble was studied and reported from Italy by Benincasa (3), Cappelluti-Altomare (12), Campbell (11), Buttaro (8), Wuiovich (55), and others.

PREVALENCE OF AND INJURY CAUSED BY THIELAVIA IN EUROPE.

Comparatively few definite data have come to the writer's attention relative to the prevalence of and the amount of injury caused by Thielavia in Italy. The trouble has been reported by Peglion

from San Sepolcro, in the vicinity of Florence, in north-central Italy; by Buttaro and Benincasa from Pontecorvo, in southwestern Italy, not far from Rome; by Bruni and Barbatelli from Scafati, south of Naples, from several other points in Italy, and from Sardinia.

Peglion gives no note of the distribution of the disease or of the amount of injury caused, but states that the mortality of the seedlings is a constant factor, shown especially in some of the more delicate and exotic varieties. Campbell (11) states that the loss caused by the rot of seedlings in the beds is known to all tobacco growers, forced as they are at times to greatly reduce their planting through lack of young plants to set out.

Benincasa (3) gives very little with regard to prevalence, but notes in 1902 that injury in the seed beds at Pontecorvo, even to the most resistant varieties, had never been as severe as that year, and adds that the disease would have reached the proportions of a disaster had not a remedy been found in the planting of later nurseries and the giving of more space to the plants. The injury in the field reached the highest limits and growth was much retarded. These reports would indicate a rather general distribution of the trouble and no small amount of injury therefrom.

HOSTS AND DISTRIBUTION.

To give some idea of the wide range of its hosts, a list of plants on which Thielavia has been found, together with the locality and authority, is given:

Plants on which Thielavia has been found, with locality and authority.

Name of plant.	Locality.	Authority.
Aralia quinquefolia (ginseng)	Ohio	Selby.
	New York	Whetzel.
Begonia rubra	Ohio	Selby.
Begonia sp	Germany	Zopf.
Catalpa speciosa	Ohio	Selby.
Cochlearia armoracia (horse-radish).	Kazan, Russia	Sorokin.
Cyclamen sp	Germany	Sorauer.
Gossypium herbaceum (cotton)	Washington, D. C	Smith, E. F.
Lupinus albus	Halle, Germany	Zopf.
Lupinus angustifolius	Halle, Germany	Zopf.
Lupinus luteus	Halle, Germany	Zopf.
Lupinus thermis	Halle, Germany	Zopf.
Linaria canadensis	Connecticut	Gilbert & Stewart.

Plants on which Thielavia has been found, with locality and authority—Continued.

Name of plant.	Locality.	Authority.
Nemophila auriculata	King's Cliffe, England	Berkeley & Broome.
Nicotiana tabacum (tobacco)	Italy	Peglion, Campbell, Buttaro, Wuiovich, Benincasa, Cappelluti-Altomare.
	Sardinia	Gafaro.
	Ohio	Selby.
	Connecticut	Shamel, Clinton, Gilbert.
	Kentucky	Gilbert.
	Cuba	Bessey, E. A.
Nicotiana rustica	(?)	Kirchner.
Onobrychis crista-galli	Halle, Germany	Zopf.
Oxalis corniculata, var. *stricta*	Connecticut	Gilbert & Stewart.
Pisum sativum (pea)	King's Cliffe, England	Berkeley & Broome.
	Halle, Germany	Zopf.
	Gembloux, Belgium	Marchal.
Phaseolus vulgaris	(?)	Kirchner.
Phaseolus multiflorus	(?)	Kirchner.
Senecio elegans	Berlin, Germany	Zopf.
Trigonella coerulea	Halle, Germany	Zopf.
Vigna sinensis (cowpea)	Washington, D. C.	Smith, E. F.
Viola odorata	Connecticut	Thaxter.
	Takoma Park, Md	Galloway.
	Washington, D. C.	Smith, E. F.
Trifolium repens (white clover)	Connecticut	Gilbert & Stewart.

In addition to the above list Aderhold (1), by means of inoculation with pure cultures, secured a limited development of the fungus on *Scorzonera hispanica*, *Daucus carota*, *Beta vulgaris*, and Apium (?), and a more vigorous growth on roots of *Phaseolus vulgaris*.

In 1905 specimens of tobacco seedlings, noted above as affected by Thielavia, were sent to this Department by Mr. Paul Ackerly, of Havana, Cuba, and the fungus was identified by Dr. Ernst A. Bessey.

SYNONYMY AND RELATIONSHIPS.

When Berkeley and Broome (5) in 1850 gave the name *Torula basicola* to the fungus which they found at the base of the stems of peas, they saw only the chlamydospore stage, which is by far the

most conspicuous as the fungus is found in nature. Though classing it as a Torula, they nevertheless seemed somewhat doubtful of its exact identity, as they state that "it is a very curious species, distinguished from most Torulæ by its articulations not being constricted."

Apparently no further observations were made on the fungus until 1876, when both Zopf (56) and Sorokin (50) report having found it, the former in Berlin in 1875 on the roots of *Senecio elegans*, and the latter at Kazan, Russia, on rotten roots of *Cochlearia armoracia* (horse-radish). Sorokin found only the chlamydospore stage, and thinking it a new fungus named it *Helminthosporium fragile*. Zopf, however, made a more complete study of the fungus, and describes two conidial forms of fructification, a pycnidium with stylospores and the perfect or ascospore stage, which placed it in the Ascomycetes. No one has since observed the pycnidia mentioned by Zopf, and as he does not note their occurrence in his later publications on Thielavia, it is to be supposed that he was mistaken in his first observations. Zopf placed the fungus in the Perisporieæ and, considering it different from existing genera, created a new genus, naming it Thielavia, after one of his favorite professors at the University of Breslau, Dr. F. von Thielau. He retained the specific name *basicola* given by Berkeley and Broome, having recognized the identity of their Torula with the second fruiting form of Thielavia.

In 1882 Saccardo (39) recorded Zopf's work, but in 1886, noting the observations of Sorokin, did not agree with him in calling the fungus described Helminthosporium, believing it to be far distant from that, and placed it in the genus Clasterosporium, though with some trepidation, as he questions whether it should not be placed among the Torulæ.

Saccardo thus failed to note the identity of Sorokin's *Helminthosporium fragile* with the chlamydospore stage of Thielavia and with Berkeley and Broome's *Torula basicola*. Sorauer (47), in the same year, was the first to discover this.

The name of the fungus, with its synonymy as now recognized, is as follows:

Thielavia basicola (B. & Br.) Zopf, Sitz. Bot. Ver. Prov. Brandenb., vol. 18, pp. 101–105, June 30, 1876.
Torula basicola (B. & Br.), Ann. & Mag. Nat. Hist., Series II, vol. 5, No. 30, p. 461, 1850.
Helminthosporium fragile Sor. Hedw., vol. 15, No. 8, p. 113, August, 1876.
Clasterosporium fragile Sacc. (Sorok.) Sacc. Syll. Fung., vol. 4, p. 386, 1886.

Nearly all writers agree in placing the genus Thielavia in the family Perisporieæ of the Perisporiaceæ, thus bringing it into close relationship with Aspergillus, Penicillium, and Perisporium and with the family Erysipheæ. Tubeuf and Smith (52) state that

this is the only genus of the Perisporieæ which causes a really serious plant disease. Jaczewski (27), however, in 1894, in an essay on the natural classification of the Pyrenomycetes, places Thielavia in the family Sphærellees and tribe Thielaviees, other genera closely related being Leptosphaeria, Trichothecium, and Ascospora.

Fischer (21) in 1897 considered Thielavia as a genus of the family Aspergillaceæ of the Plectacineæ, along with Aspergillus and Penicillium.

Only two other species of the genus have thus far been reported—*Thielavia bovina*, found growing on cow dung in Sicily, and *Thielavia soppittii*, found at the base of dead stems of *Carduus palustris* at Halifax, England.

ARTIFICIAL CULTURES OF THIELAVIA.

HISTORICAL DATA.

Peglion (33) in 1897 reported having grown Thielavia in pure culture on various sterilized media, among which were sterilized potato, must agar, and must gelatin. The fungus grew readily upon these, developing both the endoconidia and chlamydospores. The ascospores, however, Peglion was unable to obtain until three years later, when they were produced in 3-year-old potato cultures revived by the addition of a 0.6 per cent tartaric acid solution and kept in a thermostat at 25° C. for a number of days. No one has since reported the occurrence of perithecia in culture.

In the summer of 1903, Aderhold (1) undertook a series of inoculation experiments with Thielavia, and he states that the fungus grew easily on sterilized pear and carrot and on bouillon gelatin with pear and carrot juice or grape sugar.

Clinton (20) reports having grown the fungus on potato agar and on sterilized horse dung, though he had some difficulty in obtaining his original cultures.

ISOLATION OF THE FUNGUS.

The writer experienced considerable difficulty in isolating the fungus because of the fact that the chlamydospores taken from old diseased tobacco roots failed to germinate before being overgrown with bacteria and, further, because the fungus does not grow well upon plain beef agar. Cultures were finally secured from conidia obtained from freshly diseased rootlets, the medium used being a beef agar to which a 0.2 per cent solution of sodium nitrate was added.

Method used.—Sections of diseased rootlets showing little, if any, decomposition but presenting an abundance of spores were soaked for forty-five seconds in a 1 to 1,400 solution of mercuric chlorid and

then thoroughly rinsed with distilled water. Under sterile conditions they were macerated in a watch glass containing a little sterile water. Tubes of sodium nitrate agar were inoculated from this material, dilutions made therefrom, and plates poured.

CULTURAL CHARACTERISTICS.

The fungus Thielavia has been grown on a wide range of culture media and under various conditions, and exhibits considerable difference in development under changed environments.

On practically all media the mycelial growth was confined almost entirely to the surface and immediate substratum, sometimes penetrating 5 to 10 millimeters into the latter and occasionally entirely filling it with hyphæ, but producing practically no aerial growth. So far as the writer's observations have gone, endoconidia were produced aerially only, but chlamydospores have been noted to occur all through the substratum, as in the tobacco roots they are produced within the cells of the plant as well as on the surface.

In slant cultures the excess of liquid gathered at the bottom, and in all cases the growth of the fungus was quickest and most vigorous there and the production of chlamydospores most abundant.

Growth on various agars, vegetables, etc.—On beef-agar slants at the end of five weeks, during which time the cultures were kept at room temperature (18 to 24° C.), the growth was rather scant, gray except at the bottom of the slant, where it was denser and blacker and a more copious production of chlamydospores was evident. Hyphæ penetrated the agar to a depth of 7 to 10 millimeters, and chlamydospores were produced therein.

In beef bouillon the fungus produced a slightly grayish black floating mass of mycelium, sparingly spore bearing, nearly filling the liquid.

In asparagin water the growth was less vigorous and nearly transparent, very few spores being produced.

On soil-solution agar and tobacco-root agar, growth was very scant and pale gray, the few chlamydospores produced being of a very light color.

On tobacco-leaf agar the fungus covered the slant and was quite vigorous and gray-black.

On corn meal in flasks growth was very vigorous, the production of endoconidia being very abundant two to four days after inoculation and covering the mycelium with silvery masses of spores, these being hidden later by a dense black growth of chlamydospores.

When a 0.5 per cent sodium nitrate solution was added to the corn meal the fungus was even more vigorous and produced more quickly an abundance of jet-black chlamydospores.

Steamed pear slants produced a very scanty, scattered, gray-black, sparingly spore-bearing growth.

Gelatin with beef bouillon was an even poorer medium, the fungus developing but slightly on the surface.

Steamed carrot and potato slants proved the best media found, as the fungus invariably grew vigorously and produced abundantly both conidia and chlamydospores. The mycelium penetrated and blackened the substratum in both cases and formed layers of mycelial growth from the medium to the walls of the tube over the surface of the liquid.

On corn-meal agar and pear-juice agar spore production was not great, being more abundant in the former and somewhat restricted to spots, but not covering the surface of the medium in either case.

On steamed rice growth was slow and not very dense, gradually penetrating the medium and producing chlamydospores sparingly in little clumps, the conidia being equally scarce.

On 1 per cent grape-sugar agar a very dense growth was produced on the surface and penetrating 5 to 10 millimeters into the substratum. An abundance of jet-black chlamydospores was present, giving the slant a shiny black appearance.

Growth in acid media.—A 0.05 per cent oxalic-acid agar gave a very vigorous development of the fungus on the surface and penetrating 10 to 15 millimeters into the substratum, the color being a dull black. However, when 0.2 per cent oxalic acid was added to the agar no growth could be secured, though several attempts were made.

On 0.1 and 0.2 per cent nitric-acid agar, growth was gray-black, well over the slant, slightly less in 0.01 per cent and much more vigorous and more highly spore producing in 0.02 per cent agar.

Growth in neutral-salt media.—The addition of 0.1 per cent sodium sulphate to beef agar gave a sparing development of the fungus over the surface of the slant, while 0.2, 0.5, and 1 per cent added to agars produced a denser, darker colored growth, but in colonies rather than evenly covering the surface.

On 0.1 per cent potassium-nitrate agar the fungus nearly covered the slants with a gray-black growth, but on 0.2 and 0.5 per cent agar, development was denser and blacker, in the latter case being in colonies.

On 0.1 and 0.2 per cent sodium-nitrate agar eventually an abundant growth was produced, but on 0.5 and 1 per cent the development was more scant and spore production not as great.

Growth in alkaline media.—On 0.1 and 0.2 per cent potassium-carbonate agar a spreading, scant, gray to black growth was produced, while agar containing 0.5 and 1 per cent potassium carbonate gave no development of the fungus whatever.

A 0.5 per cent calcium-hydroxid agar gave slightly more vigorous growth than an agar containing 1 per cent of the salt.

Summary.—Summarizing the results here obtained, it would seem that the presence of the various acids and salts up to a certain point, different in the case of each ingredient used, is favorable to the growth of Thielavia, while beyond that point it tends to retard, restrict, or even kill the fungus.

Moreover, the abundance and color of the chlamydospores varies with the medium employed. Oxalic-acid and grape-sugar agars and corn meal plus 0.2 per cent sodium nitrate gave notably jet-black chlamydospores in profusion, while pear and potassium-carbonate agars and pear slants produced light gray to olive colored spores.

EFFECT OF DIFFERENT TEMPERATURES.

Differences in temperature also produced marked variations in growth, especially with regard to rapidity of spore formation. Cultures of Thielavia on carrot slants, kept in an ice box at a temperature of from 8° to 11° C., grew very slowly, at the end of the third day showing only a very faint white growth along the line of inoculation, and the first sign of chlamydospore development appearing after six days.

The lower limit of growth was determined by means of the ice thermostat to be between 7° and 8° C. No growth was made in cultures kept at 1.3° C., but when these were brought into room temperature vigorous growth resulted.

At room temperature, 18° to 24° C., after two days a white, cottony growth covered two-thirds of the slant, spreading from the line of inoculation, and chlamydospores began to appear the third day.

In the thermostat at 30° C. spore formation was slightly earlier and growth considerably more vigorous and rapid. This temperature was clearly the optimum for the development of the fungus.

In cultures kept at 34° C. a curious phenomenon occurred. The fungus, instead of spreading over the entire surface of the carrot slant, as in other cases, confined its growth to a limited space adjacent to the line of inoculation, producing a dense, fluffy, gray-black mass of mycelium and spores, 2 to 6 millimeters wide along the length of the inoculation and heaped above the surface of the medium to a height of 1 to 3 millimeters, in this particular being quite different from any other cultures.

At 37° C. no growth could be secured on carrot slants. The upper limit of growth, then, is between 34° and 37° C.

SPORE GERMINATION.

Germination of both endoconidia and chlamydospores was observed in hanging-drop cultures of beef bouillon. The former germinated

in eighteen hours, and in one case a spore after putting out a single hypha six cells long began the production of new conidia in large numbers, the last cell of the six functioning as an endoconidial cell. (Pl. I, figs. 2 and 3.)

The chlamydospores were from an old tobacco root and germinated after eighteen to twenty-four hours. (Pl. I, fig. 7.)

CONDITIONS INFLUENCING ATTACKS OF THIELAVIA.

The conditions prevailing in tobacco seed beds are so different from those encountered in the field, and the possible means of prevention of root-rot in the two cases are so distinct because of both the limited seed-bed space and the diverse conditions under which the plants grow, that the subjects will be treated separately.

CONDITIONS IN THE SEED BEDS.

In so limited a space as the tobacco seed beds, especially those in northern tobacco districts made in the most up-to-date manner with steam heat and sash covers, the various environmental factors are very largely under control, and preventive measures can be more easily carried out than in fields.

GENERAL CONCLUSIONS OF VARIOUS WRITERS.

Sorauer (48) in his study of the "root-brown" of cyclamens observed that certain conditions, especially heavy manuring, too abundant watering, and too high temperatures seemed to favor the attack of Thielavia, and he states that by changing the affected plants to a sandy, less rich soil and giving them abundant ventilation, plenty of sunlight, and not too much water, an increase of the disease was prevented. Peglion (33) further states that in hotbeds the stratum of manure may be so near the surface as to injure the roots and to predispose them to the attacks of Thielavia.

Benincasa (3), while recognizing these facts, believed that "more than all, certain weather conditions favor the development of the parasite. When the season is humid, the environment in which the young plants are compelled to live, while predisposing them to the attacks of disease, is extremely favorable to the development of the fungus. Scarcely any expedient is successful under such circumstances, either in preparing or cultivating the nursery." In addition to the measures recommended by Sorauer (48) for checking the trouble, Benincasa (3) notes the use of a 0.6 per cent KNO_3 solution for watering the plants, to stimulate the development of new secondary roots. On the other hand, solutions of manure aggravate the trouble.

RESULTS OF SEED-BED EXAMINATIONS.

In the writer's experience the worst root-rot has been found in seed beds containing soil with an abundance of humus and a considerable percentage of clay, highly fertilized either with chemicals or manure, and excessively watered. On the other hand, many seed beds with a light sandy soil containing little humus and not highly fertilized have been examined, and with very few exceptions no root-rot has been found.

EXCESSIVE FERTILIZATION.

The fact that an abundance of humus and quickly available nitrogenous fertilizers, high temperatures, and abundant watering will produce large plants in the shortest space of time has led many to use these conditions of excessive and rapid growth in the production of tobacco plants, often going to the extreme in the quantities of fertilizer and water used. The result is that the plants are made weak and especially susceptible to the attacks of Thielavia, while at the same time ideal conditions are furnished for the growth of that parasite. Many cases of serious seed-bed injury observed by the writer were connected with excessive fertilization; in some instances with chemicals containing large quantities of quickly available nitrogenous ingredients, such as nitrate of soda; in others, large quantities of hen manure were used. In addition to this fertilization previous to seeding, the practice is quite prevalent of watering the young seedlings from time to time with dilute solutions of $NaNO_3$ or manure water to hasten growth, and this in all probability aggravates the trouble.

VENTILATION.

The ventilation of the seed beds also has an effect upon the development of root-rot, though just how great it is difficult to determine because of the large number of factors which are to be considered.

Sash compared with cloth-covered beds.—It was thought at first that the disease would be found much more severe in beds covered with sash than in cloth-covered beds, because of the fact that the air becomes humid and heated by the sun, thus furnishing ideal conditions for the development of the fungus. The results of the examination of a considerable number of diseased seed beds, both sash and cloth covered, bears out this inference with very few exceptions. Nearly all of the worst diseased beds were glass covered, and the number of diseased sash-covered beds as compared with cloth-covered ones was more than two to one.

In one case where a cloth-covered bed was more severely attacked than an adjoining sash-covered one, though the disease was bad in

both, the former had been diseased quite badly the previous year and thus was very heavily infected with the fungus, while the latter was attacked for the first time and the infection was consequently not as great.

Benincasa (3) states that he has seen root-rot develop in its most virulent form in seed beds covered with glass or oiled paper. All things considered, it seems reasonably sure that sash-covered beds unless very carefully ventilated are liable to more serious attacks of root-rot than are cloth-covered beds.

Crowding of plants.—The crowding of plants in the seed beds by too heavy seeding furnishes favorable conditions for the development of root-rot, at the same time causing the seedlings to be spindling and tender and thus more susceptible to the disease. This results not only from the lack of sunlight and a free circulation of air about the plants themselves, but because the dense mass of foliage of the crowded plantlets produces a damp blanket over the soil and prevents proper aeration and circulation, thus aiding fungous development.

Too heavy seeding of tobacco beds is a common fault and not altogether without cause, for the reason that tobacco seeds are so very minute that it is difficult to sow a small enough quantity. Moreover, if any wind is blowing, the seeds are apt to be unequally distributed, resulting in crowding in some spots, while others are nearly or quite bare. The best method to avoid crowding and to insure even seeding is to mix the seed thoroughly with several times its bulk of meal, ashes, plaster, dry sand, or soil and go over the bed twice, once lengthwise and the second time crosswise, sowing as small a quantity as possible each time. After a little experience with this method of sowing, even distribution of the seed can be obtained and too heavy seeding prevented, thus insuring more vigorous plants.

Benincasa (3) states that in 1902, at the tobacco agency at Pontecorvo, Italy, excessive injury from root-rot was avoided, even though the disease was more than ordinarily severe, by sowing the seed beds later and giving the plants more space.

TEMPERATURE.

The few data available from actual practice have not shown that steam-heated, sash-covered beds are more susceptible to root-rot than those which rely solely on the sun's heat. One large group of steam-heated, sash-covered beds was examined quite carefully in the spring of 1906 and comparatively little root-rot found in two or three beds. The soil, however, was of a medium light texture, and therefore not especially favorable to the development of the trouble.

Severe winters.—By some the virulence of root-rot is thought to be measurably affected by the character of the preceding winter, a severe season causing a considerable diminution of the trouble, but no direct evidence is available on this point, as no experiments have been carried out to determine the effect of freezing on the viability of the spores. However, the evidence at hand from practical men supports the statement.

SEVERE DRYING.

The result of long-continued desiccation of seed beds during the summer was in one case an increase of root-rot the following spring. As an experiment one farmer left the sash on a portion of one of his worst diseased beds all summer. The soil became so dry that every weed in the bed died and the earth was baked by the sun all summer long. The following spring the bed was prepared as usual, and the disease appeared even worse than in the previous season.

CONTINUOUS USE OF BEDS.

The result of using seed beds year after year without sterilization or change of soil is an increase of root-rot. If the bed is infected at a particular point and the conditions are favorable, the fungus will spread by growth through the soil and be scattered in the spading and preparation of the beds in the spring, so that in the course of a year or two at the most the entire bed will become infected.

NEW SOIL IN OLD BEDS.

By taking the infected soil from the beds to a depth of 10 to 12 inches and supplying fresh soil, a fairly good crop of seedlings has in some cases been grown the first year, but the spores of the fungus left about the sides or in the soil below were sufficient to infect the new soil, and by the second season at the latest the disease had reappeared in serious proportions. In one instance, however, that came to the writer's attention, new soil was added in part and mixed with old soil where the trouble was severe, with the result that the disease caused serious loss the first season.

NEW BEDS.

Even the preparation of entirely new beds every season does not insure immunity from attack, as the fungus may be introduced in so many different ways that it is almost impossible to guard against it by other means than soil sterilization, especially in sections where the disease is at all common.

GREENHOUSE EXPERIMENTS.

EFFECT UPON ROOT-ROT OF HEAVY AND OF LIGHT WATERING AND OF A SURFACE LAYER OF SAND.

Four large flats were filled with rich soil. Three were artificially inoculated with Thielavia by mixing a number of vigorous corn-meal cultures with the soil; one was left uninoculated as a check. All were kept at greenhouse temperature. Flat A was watered excessively, flat B scantily—just enough to keep the plants from wilting—and flat C received a moderate amount of water and a surface layer of sand one-half inch deep.

The experiment was terminated six weeks after sowing the seed, and out of 587 plants examined in flat A every one was badly affected with Thielavia. The disease, while present in all the remaining flats, including the check, was in no case as severe as in flat A. Next to the check, flat B showed the smallest amount of disease, 62 per cent of the plants being quite badly attacked and the remainder but slightly. In flat C none of the roots in the sand were diseased, save where the fungus had run up on a root from the infected soil below. However, a large percentage of the roots which entered the soil below were more or less diseased.

Conditions in the Fields.

CLAY SOIL COMPARED WITH SANDY LOAM.

Root-rot in the field causes by far the greater loss on soils containing a large percentage of clay and lacking in drainage; in some cases the entire crop is rendered unmarketable.

In the medium light, sandy loams the injury, though often considerable, rarely, if ever, amounts to a total loss of the crop. The loss in these cases is due to the necessity of having to reset a larger percentage than usual of plants that were too weak and were overcome by the attacks of disease, and to a retarding of the development of the affected plants, thus resulting in a reduction in the yield and in injury to the quality of the crop. In some instances the retarding of the newly set plants has been such as to cause farmers to plow up whole fields and to reset them with healthy plants. In other cases, however, the loss is hardly appreciable, though some delay is noticeable in the starting of the crop.

LOW SPOTS.

The greater injury to tobacco in low spots in fields is attributed by some growers to the burning of the roots by an excess of some constituent of the commercial fertilizers used which by washing and seepage has accumulated there. A grayish white incrustation was

found quite general on the soil in badly diseased spots. An analysis of this made by the Bureau of Soils, in comparison with soil from 2 to 4 inches below the surface and soil from a healthy portion of the same field at a similar depth, gave a very large excess of soluble nitrates, sulphates, and chlorids in the first case and a considerable increase in the second above the content of healthy soil, the percentages of these soluble salts being, respectively, 0.7 per cent, 0.2 per cent, and 0.06 per cent. Professor Whitney, who made the analysis, further states that this examination would indicate that an excessive accumulation of soluble salts is the probable cause of the trouble with the tobacco plants grown on this soil.

The presence of this excessive amount of soluble salts may be injurious to the plant roots and thus render them more susceptible to the attacks of the fungous parasite. The cultural experiments described have shown that Thielavia grows more vigorously in the presence of 0.2 per cent of various salts than in the presence of smaller amounts, and therefore that the excessive use of fertilizers also favors the growth of the fungus.

HIGH FERTILIZATION.

Just what relation the high fertilization of tobacco fields in vogue in many places has upon the development of root-rot can not be answered conclusively at this time, though work is being done along this line by Dr. L. J. Briggs, of the Bureau of Plant Industry. As the result of fertilizer experiments already completed, he concludes (6) that the root-rot "fungus attacks the tobacco roots most severely when the soil has become alkaline, due to the use of too large amounts of lime, ashes, or fertilizers containing carbonate of potash."

ROTATION OF CROPS.

Numerous field observations have been made on the effect of crop rotation upon the virulence of root-rot of tobacco. In a field of a number of acres comprising one portion previously in tobacco and another which had been in corn, there was a distinct line of demarcation between the two portions of the field—that previously in tobacco having the disease much worse than the other, the plants being stunted and sickly and exhibiting considerable Thielavia on their roots, while tobacco on the corn land was larger and more vigorous and healthy in appearance and was little affected by Thielavia. The same marked difference has been noted between old tobacco fields and new lands adjoining just brought into cultivation. The growing of grass on tobacco land for one year has likewise caused a striking decrease in the amount of root-rot and an increase in the vigor of the resulting crop.

In one instance a field which had been in tobacco continuously for a number of years and showed considerable root-rot was enlarged by including a strip of land 5 to 15 feet wide on two sides which had not been cultivated but had borne a growth of weeds. The tobacco on this fringe was distinctly larger and more vigorous and had less Thielavia.

EXPERIMENTS.

Result of setting diseased plants in the field.—A point of great practical importance to the farmer with diseased beds is to know whether or not the injured plants may be set in the fields with fair prospects of a crop. Benincasa (3) says that the transplanting of the young diseased plants does not insure success in the cultivation, and observations by the writer bear out the statement with few exceptions.

To determine the effect of setting diseased plants in a field free from the disease, the following experiment was carried out with the assistance of Mr. J. B. Stewart, of the Bureau of Plant Industry:

Four hundred and fifty Havana Broadleaf plants, every one affected in some degree with root-rot, were selected from a diseased bed, and a like number of perfectly healthy plants of the same variety was secured from another source. These were set by machine in two long rows side by side in a field of medium light, sandy loam, the healthy plants being set first to avoid danger of infection. The diseased row was at first noticeably retarded, no growth being apparent for a period of ten days to two weeks. The plants finally gained the ascendency over the disease, and, although slightly behind the healthy row for most of the season, caught up at the last, and shortly before harvesting time little, if any, difference was apparent in either the size or the vigor of the plants. The diseased row yielded 1 pound more of cured tobacco than the healthy one and from a smaller number of plants, as quite a number of mosaic plants were discarded from the diseased row. The mosaic disease was much worse in the diseased row than in the healthy one. However, we have no conclusive evidence that the presence or absence of root-rot has any connection with the virulence of the mosaic disease. Upon examination of the root systems of full-grown plants from the diseased row, the lower portion of the taproot was found to be shriveled, black, and dead from Thielavia attacks, but a vigorous production of lateral roots which were perfectly healthy and showed no signs of root-rot had taken place above.

Although in point of yield the two rows may not be strictly comparable, as the plants were doubtless of slightly different strains, though of the same variety, and were also from different sources, the experiment showed that under the particular conditions in which the plants were grown the disease did little appreciable damage.

An instance with a like result came to the writer's notice during the season of 1906. A farmer pulled from his beds sufficient plants to set half an acre, but upon finding them so badly diseased that the root systems were largely rotted off decided not to set them, placing the seedlings in the cellar instead. After three or four days the plants developed new roots, and they were set in one corner of a large field of quite light, sandy loam. They made no growth for ten days to two weeks, and healthy plants set in the adjoining field some days later started off much more rapidly. However, at the end of the season no appreciable difference could be noted in either vigor, yield, or quality. The roots exhibited the same appearance as in the case already noted.

Result of setting healthy plants in infected soil.—A second experiment was made on a smaller scale in which diseased seedlings were set in a noninfected field and healthy ones in a diseased seed bed after the removal of the seedlings. In the first case the result was almost identical with the experiment already noted, the soil being very similar. In the latter case, however, the healthy seedlings became quite badly diseased, were notably stunted, and a large percentage failed to produce mature plants that were marketable.

In the greenhouse sixty-six plants of Connecticut Havana tobacco were grown in pots of uninfected soil until 4 to 6 inches high, and then twenty were inoculated by mixing some infected soil from a Connecticut seed bed with the soil in which they were growing. The remaining plants were kept for checks. At the termination of the experiment the inoculated plants were without exception from 4 to 6 inches shorter than the check plants and showed considerable root-rot besides a general unhealthy appearance.

Conclusions.—While these experiments would indicate that sometimes diseased plants may be set in the field without danger of loss, the writer would not recommend such action. All the soils in which these experiments were carried out were of very light texture and unfavorable to the development of root-rot. On heavier soils the disease has caused large losses, due both to the stunting and to the killing of the plants. Moreover, the initial retarding of the plants delays maturity, and delayed maturity always affects the quality of the crop injuriously. It also increases the danger of the crop being caught by frost. In a large field the percentage of plants that have to be reset because of irreparable injury due to Thielavia is in most cases much larger than ordinarily, while in a number of cases that have come to the writer's attention whole fields have been plowed up and reset with healthy plants.

REMEDIAL TREATMENT IN THE SEED BED.

Sorauer (48) seems to have been the first to devise methods of reducing the injury from Thielavia, the host attacked being in his case the cyclamen. Since that time Selby (44) and Clinton (20) in America and several workers in Italy have experimented with methods of reducing or eliminating the loss occasioned by Thielavia in tobacco beds.

The measures which may be employed may be divided into two classes, preventive and palliative. The former relates to all methods of eliminating the disease by killing the fungus which occasions it. Under the latter are included all those means which tend to reduce loss because of the partial or total elimination of conditions of soil, air, and temperature favoring the development of the fungus or because of the production of plants resistant to the disease.

PREVENTIVE MEASURES.

STEAM STERILIZATION.

The preventive method which promises best results to those who have the conveniences for applying it is that of sterilization of the seed beds by steam.

In addition to the killing of the fungus, this method, in common with surface firing, to be described later, has several advantages over formalin treatments. The weed seeds in the soil are very largely killed, and this alone, according to the testimony of the farmers who have used sterilization, pays for the cost of treatment, as the beds do not have to be weeded and thus a large amount of hand labor is obviated. The physical texture of the soil is altered by the heat and made more suitable to root development and, moreover, considerable plant food is made directly available to the seedlings. Furthermore, the heating of the soil just before sowing in the spring has an appreciable effect in starting the seedlings off quickly.

With the elimination of the fungus it is possible to employ those methods of forcing the plants by extra fertilization, increased watering, and higher temperatures which would otherwise be unsafe as favoring the development of the root-rot fungus.

Ordinary greenhouse method.—The method of sterilization to be used will depend to some extent on the size, the location, and the permanency of beds and the cost of application.

The method in general use for the sterilization of soil in greenhouse benches might advantageously be employed in beds that are to be used year after year without change of location, as the equipment would be more or less permanent. This consists in placing 1

foot below the surface of the soil a system of 1½-inch pipes which are perforated with ¼-inch holes on their under side at intervals of 6 inches throughout their entire length. The pipes should run lengthwise of the bed, 18 inches apart, and be connected with a steam boiler capable of producing 80 to 100 pounds pressure. Before treatment the soil should be thoroughly spaded up and pulverized to permit ready access of the steam to all parts, and all fertilizers except commercial ones should be applied at this time, since fresh spores of the fungus might be carried in if manure were added after sterilization. Commercial fertilizers, however, contain no Thielavia spores and may be applied subsequently. Preferably, however, they should be applied before sterilization and the seed sown the following day.

The bed to be treated should be covered with several thicknesses of old burlap or blankets to confine the heat to the soil. The steam should be applied at a pressure of 80 to 100 pounds, as at high pressure it is much drier and the soil is not wet as much as when low-pressure steam is used. A treatment of from one to two hours is usually sufficient to thoroughly sterilize the soil to a depth of 18 inches. A few potatoes laid in the surface soil will indicate the thoroughness of the treatment by the degree to which they are cooked. The blankets might advantageously be left on for some time to make the treatment more thorough.

While this method offers some advantages for seed beds of limited area, in that the pipes may be left in the ground and used year after year with little extra labor and may also be used for subirrigation, the initial cost of installation, especially on large seed-bed areas, may be prohibitive.

Steam-rake method.—The method of sterilization by a steam rake has not proved successful in practice. The implement is in the form of a rake made of hollow, perforated pipes, which are forced into the thoroughly pulverized seed-bed soil and through which the steam is applied. The difficulty is that the steam, instead of permeating the soil, follows the path of least resistance and escapes up the side of the teeth.

Inverted-pan method.—The method which has given the best results in practice, and which because of its simplicity and small cost recommends itself for use on large or small areas, is the invention of Mr. A. D. Shamel, of the Bureau of Plant Industry, and was devised by him to sterilize nematode-infested soils in Florida. The apparatus consists of a galvanized iron pan, 6 by 10 feet and 6 inches deep, which is inverted over the soil to be sterilized and the steam admitted under pressure. The pan is supplied with steam hose connections, has sharp edges, which are forced into the soil on all sides to prevent the escape of steam, and is fitted with handles for moving

it from place to place, the weight of the entire pan being not over 400 pounds.

The soil is prepared as in the greenhouse method, a few potatoes being buried at a depth of a foot to gauge the degree of heat attained. A soil thermometer may also be used if desired. The steam should be kept at as high a pressure as possible, 80 to 100 pounds being best, and the treatment should continue for one to two hours, depending on the pressure maintained. In experiments conducted in the spring of 1907, one hour's steaming at 80° C. under 100 pounds pressure gave best results in killing both the fungus and the weed seeds. When one section of the bed is treated the pan is lifted and carried to an unsterilized portion and the operation repeated until the entire bed is steamed. (Pl. V, fig. 2.)

SURFACE FIRING.

The surface firing of seed beds has been a common practice for years in some tobacco sections, particularly Kentucky and the South, the end in view being the improving of the tilth of the soil and the killing of weed seeds rather than of any fungous disease, though in all probability this custom accounts for the fact that little trouble is known to occur in such sections from root-rot. In Italy, as well, burning is now considered a part of the regular method of seed-bed preparation; it having been resorted to as a preventive of root-rot.

Direct firing.—Two methods of surface firing are in vogue, the first by direct firing and the second requiring the use of a pan. In direct firing, the land to be sterilized is first thoroughly pulverized and manure applied. It is then covered with straw, brush, and wood sufficient to make a hot fire. This is ignited and allowed to burn for an hour or so, and then moved along to a new adjacent spot. The ashes are raked into the surface soil and the seed is sown.

Pan firing.—The second method consists in the use of a sheet-iron pan, 3 by 9 feet, under which a fire is made. This is set in the middle of a 9-foot bed and the soil on one side to a depth of 6 inches is shoveled in and heated, great care being taken to keep it moist, otherwise the humus would be burned out and the physical texture irreparably altered. After an hour this soil is put back and that from the other side of the pan subjected to the same treatment and then the pan moved along to a new place. The soil underneath the pan itself is thereby subjected to heat for two hours.

FORMALIN STERILIZATION.

The use of a formalin solution for the sterilization of greenhouse soil and of tobacco seed beds against Rhizoctonia has been in vogue for some time with excellent results, and furnishes a very simple

means of combating the root-rot. The method is as follows: The beds are thoroughly prepared the same as for the other methods of sterilization described and are then drenched with a formalin solution composed of 1 part of commercial formalin to 150 to 200 parts of water, three-fourths to 1 gallon of this solution being used to the square foot of bed space. The solution should be put on with a watering pot with a rose and distributed as evenly as possible over the bed, so as to thoroughly wet the soil to the depth of a foot. It will in most cases be necessary to put this solution on in two or three applications, as the soil will not take in this quantity of water immediately. The beds should then be covered with heavy burlap or a tarpaulin to keep in the fumes for a day or so, and then aired for a week before sowing the seed.

Spring applications of formalin are open to the following objections: The addition of such a large quantity of water to the soil keeps it wet and cold for some time longer than would naturally be the case, thus delaying germination as well as subsequent growth; the necessity of airing the beds to remove the formalin fumes and to allow the soil to dry out also causes delay in seeding. To obviate this difficulty the beds should be treated in the fall, before freezing weather sets in. In this case a stronger solution, 1 to 100, may well be used, as there will of course be no danger then of injuring the seedlings.

COMPARATIVE EXPERIMENTS WITH STEAM AND FORMALIN STERILIZATION.

Numerous greenhouse experiments have been made to determine the comparative merits of steam and formalin sterilization, and a seed-bed trial was carried out in cooperation with Messrs. A. D. Shamel and J. B. Stewart, of the Bureau of Plant Industry, involving a comparison of steam sterilization, surface firing, and formalin drenching as means of preventing the tobacco root-rot.

Steam sterilization.—To determine the effect of different degrees of heat and different lengths of steam treatment of soil upon the development of Thielavia the following experiment was made: Six 8-inch pots were filled half full of good greenhouse soil, and sufficient soil to fill them was added from a badly diseased tobacco seed bed in Connecticut. They were then autoclaved one and one-half hours at 20 pounds pressure at 126° C. On the following day four of these were again autoclaved in the same manner, and two again on the third day. Two unsterilized 10-inch pots of the same infected soil were used for checks, and all were sown on the same day with Connecticut Havana seed. Germination was rather uneven in all the pots. After three weeks a considerable number of seedlings in the check pots damped-off with Thielavia, but those in the steam-treated pots were

perfectly healthy, and a week later were two to three times as large as the survivors in the check pots. These remained much smaller and were sickly in appearance throughout the experiment. (Pl. IV, fig. 3.)

At the end of eight weeks the plants in all the pots were pulled and found in the following condition:

Check pots: Plants distinctly stunted and foliage a sickly yellow color characteristic of root-rot. Roots of all plants badly diseased with Thielavia.

Pots autoclaved one day: Plants deep green, vigorous, and healthy. A small amount of Thielavia on the roots of one plant.

Pots autoclaved two days: In one pot no germination occurred; in the second pot the plants were quite vigorous and healthy and no Thielavia was found.

Pots autoclaved three days: Plants fairly vigorous and healthy, though somewhat crowded. Root systems perfectly healthy.

The result of this experiment was that in five sterilized pots one plant only was slightly attacked by Thielavia (doubtless owing to chance reinfection after sterilization), while every plant in the two untreated pots was badly diseased.

The experiment was repeated with the same pots, the soil being reinfected by mixing it with infected soil and cut-up, diseased tobacco roots and allowed to stand two days before treatment.

Two pots were autoclaved one and one-half hours at 102° C., 1 pound pressure; two pots were autoclaved one and one-half hours at 108° C., 5 pounds pressure; two pots were autoclaved one and one-half hours at 126° C., 20 pounds pressure; two pots were left for checks.

All pots were sown on the same date with Connecticut Havana seed. Germination was good in all. As in the previous experiment, by the end of the fifth week a very large majority of the plants in the check pots had been killed by the attacks of Thielavia, only a few scattering, stunted plants remaining, while in the sterilized pots no signs of Thielavia were to be seen. At the end of eight weeks plants were pulled from the sterilized pots, and the roots were found perfectly healthy. When the few plants left in the check pots were pulled, all were found to be diseased.

Formalin sterilization.—To test the effect of different strengths of formalin solution used as a drench to prevent root-rot, the following experiments were made:

Three boxes 10 by 32 by 6 inches were filled with rich potting soil and thoroughly inoculated with Thielavia by mixing with the top 2 or 3 inches of soil a considerable quantity of a pure culture of Thielavia on corn meal. One was kept for a check, and the other two were treated the next day with formalin solution as follows: Box A was drenched with a 1 to 200 formalin solution, three-fourths gallon being used to the square foot of surface; box B was treated in like manner with a 1 to 300 formalin solution. The boxes were

allowed to stand for several days to permit the formalin fumes to escape and the soil to dry out.

At the end of two weeks the plants in the treated boxes were healthy and vigorous, while the check showed poorer germination, the plants were not as vigorous, and some Thielavia was to be found.

At the expiration of three weeks the plants in the treated boxes had continued their vigorous growth, while the check showed plants small, much diseased, scattered, and sickly in appearance. Many were dead from root-rot. The experiment was terminated at the end of six weeks and the plants pulled and examined (see Pl. III, fig. 3), with the following results:

Check box: Plants very scattering, sickly green in color, 1 to 2 inches tall; 148 plants examined and every one found diseased with Thielavia.

Box A, treated with 1 to 200 formalin solution: Plants vigorous and healthy in appearance, 5 to 7 inches tall. About 500 plants examined and Thielavia found on the roots of one plant; roots of the remainder perfectly healthy.

Box B, treated with 1 to 300 formalin solution: Plants healthy in appearance, 6 to 8 inches tall. Out of 268 plants examined 93 were found perfectly healthy and 175 diseased by Thielavia—some very slightly, some badly.

The experiment was repeated with slightly different results, as follows:

Check box: Plants very badly diseased with Thielavia.

Box A, treated with 1 to 200 formalin solution: Considerable Thielavia present.

Box B, treated with 1 to 300 formalin solution: There was a very little Thielavia on 8 roots out of the large number of plants in the box, the remainder being entirely healthy.

The disease in box A may have been due to reinfection from the check or from some other source. Owing to the absence of the writer the experiment was run much longer than it should have been, and the plants being excessively crowded in the box made a very weak, spindling growth, thus rendering them especially susceptible to the attacks of Thielavia.

Seed-bed trial of sterilization methods.—To test thoroughly the steam and formalin methods of sterilization, together with that of surface firing, under actual seed-bed conditions, the following experiment was carried out in the spring of 1907 at Simsbury, Conn., in a seed bed which had been badly diseased the preceding year. (Pl. IV, fig. 1.)

It should be stated at this point that while the plans were outlined by the writer, all material from the several beds examined by him from time to time, and the conditions of the beds noted at the termination of the experiment, the work of preparation, treatment, and subsequent care of the seed beds was done by Mr. J. B. Stewart, owing to the fact that other work prevented the writer from giving the experiment personal attention.

A diseased bed, 62 feet long and 6 feet wide, was divided into 7 sections, which received different treatments, as illustrated in the following diagram:

Section 1 (10 feet).	Section 2 (8 feet).	Section 3 (8 feet).	Section 4 (8 feet).	Section 5 (8 feet).	Section 6 (10 feet).	Section 7 (10 feet).
Open wood fire. 1 hour.	Check. Untreated.	Formalin, 1 to 200. Three-fourths gallon per square foot.	Formalin, 1 to 300. Three-fourths gallon per square foot.	Steam. Soil heated to 200° F. for ½ hour.	Steam. Soil heated to 175° F. for 1 hour.	Steam. Soil heated to 150° F. for 1½ hours.

The soil was thoroughly spaded up and fertilizers added before treatment. The sections were separated by 1-inch boards inserted several inches into the soil and extending 4 to 6 inches above it. The various sterilization treatments were given April 15 and the Havana Broadleaf seed put to sprout in moist apple-wood punk and sown four days later. The beds were then covered with cheese cloth, which was kept on during the early part of the experiment until warm weather set in.

On section 1 a fire of brush and wood was burned for an hour and the ashes raked into the topsoil.

On sections 3 and 4 the formalin solution was applied with a watering pot with a rose. The solution was put on in several installments, as the soil would not take it all up at once.

Sections 5, 6, and 7 were steam sterilized by the inverted-pan method (described on p. 35). Section 5 was treated for a half hour after the soil had reached a temperature of 200° F. as indicated by a soil thermometer inserted 6 to 8 inches below the surface of the soil. Section 6 was treated one hour at a temperature of 175° F., and section 7 one and one-half hours at a temperature of 150° F.

The following notes made by Mr. Stewart give the condition of the beds on the dates stated:

April 26. Plants coming up on all steam-sterilized and check sections, but none showing on formalin or open-fire burnt sections.

April 30. Plants on all steam and fire sterilized and check sections up and growing nicely, but plants on formalin-treated sections are not coming up: only very few plants can be found, and these are small and weak.

May 6. Plants on all sterilized beds except formalin treated doing nicely. Steam-sterilized and check sections look best; plants are largest. Then comes the open-fire section. On formalin sections plants just coming up.

May 14. Plants all doing very nicely for cloth beds. Steam-sterilized and check sections about same in advancement, and fire and formalin treated about the same. Formalin-treated beds brisking up, though plants are thinner than on other sections.

May 21. In steam-sterilized sections plants too thick; in formalin-treated sections too thin; check and fire-sterilized sections about right. Steamed sec-

tions at least one week ahead of others. These were thinned and whole bed fertilized with one-third pail of a special fertilizer and well watered down. No disease showing as yet.

On May 30, plants from several beds were sent by Mr. Stewart to the writer, who examined them with the following results:

Section 7, steam sterilized one and one-half hours at 150° F. No disease.
Section 1, open-fire sterilized. A few plants slightly diseased.
Section 3, formalin treated, 1 to 200. One plant slightly diseased.
Section 2, check. A few plants diseased.

Mr. Stewart's notes continue as follows:

June 7. Plants in steamed sections ready to set; show no disease. In formalin-treated sections, one week behind. No disease yet definite. Check, all plants diseased.

On June 12, about 100 plants from each bed were pulled and sent to the writer, who examined them with the following results:

Section 1, fire sterilized. Plants 2 to 3 inches tall, healthy in appearance. No Thielavia found.

Section 2, check. Plants one-half to 2 inches tall, stunted and badly diseased. Thielavia abundant.

Section 3, formalin treated, 1 to 200. Plants 3 inches tall. Thielavia found on quite a number, though not bad as yet.

Section 4, formalin treated, 1 to 300. Plants 2 inches tall. Thielavia found on a considerable number; none bad enough to be noticeable to the naked eye without careful examination.

Section 5, steamed one-half hour at 200° F. Plants 2 to 3 inches tall, healthy. A very small amount of Thielavia found. The great majority of roots perfectly healthy.

Section 6, steamed one hour at 175° F. Plants 3 to 4 inches tall, healthy. No Thielavia found.

Section 7, steamed one and one-half hours at 150° F. Plants 4 to 6 inches tall, healthy, and with good root systems. Practically free from Thielavia, though a few rootlets were found showing the trouble in slight measure.

The following notes are also by Mr. Stewart:

June 20. Five thousand plants pulled for setting from three steamed sections.

June 27. Eleven thousand plants taken from same sections and some from open-fire section. No plants were pulled for setting from either formalin-treated or check sections.

On July 9, about 100 plants were pulled from each bed and sent to the writer, who examined them with the following results:

Section 1, fire sterilized. Out of 106 plants examined, 40 were perfectly healthy and 66 diseased—a few quite badly, the majority moderately.

Section 2, check. Ninety-nine plants, all diseased from moderately to very badly.

Section 3, formalin treated, 1 to 200. Eighty-five plants badly diseased.

Section 4, formalin treated, 1 to 300. Ninety plants, all more or less diseased.

Section 5, steamed one-half hour at 200° F. Sixty plants badly diseased; 25 slightly, if at all.

Section 6, steamed one hour at 175° F. Seventy plants healthy, 13 slightly diseased.

Section 7, steamed one and one-half hours at 150° F. Plants very healthy in appearance, 80 with healthy root systems, 20 slightly diseased.

On July 12 the beds were examined by the writer and found in the following condition, which also summarizes the results of the trial, the sections being treated in the order of their excellence. None of the beds were weeded during the experiment.

Section 6, steamed one hour at 175° F. Best bed in every respect, all plants dark green and healthy, almost entirely free from disease. Hardly a weed in the plat. (See Pl. IV, fig. 2.)

Sections 5 and 7, steamed one-half hour at 200° F. and steamed one and a half hours at 150° F. About the same in appearance as section 6, although slightly more disease present in both plats, but not enough to do any real harm. A very few weeds were left in both beds.

Section 1, surface-fire sterilized. Not as healthy in appearance as steamed sections. Considerable disease to be found all over the plat. Somewhat weedy.

Section 4, formalin treated, 1 to 300. Disease found on most plants, sometimes quite bad. Plants sickly in appearance. Badly overgrown and choked by weeds.

Section 3, formalin treated, 1 to 200. Disease found on every plant; on some very bad. Choked by weeds; even worse than section 4.

Section 2, check. Plants very scattering, many dead, very badly overgrown by weeds. Remaining plants all more or less diseased, many very badly.

To summarize, the steam treatments gave excellent results, the surface-fire treatment fair returns, while the two formalin-treated plats were little better than the untreated check plat.

Several reasons may be advanced to account for the failure of the formalin treatment. The seed was probably sown too soon after the treatment, so that the fumes of formalin had not fully escaped from the soil, and retarded and injured the germinating seedlings. Moreover, the soil had not dried out sufficiently, so that the bed was wet and cold. Both of these conditions would tend to produce weak plants easily attacked by the fungus from the adjoining check plat, which was separated by a board partition only. Fall application of the formalin would obviate both of these difficulties and, moreover, a stronger solution, 1 to 100, could advantageously be used with greater certainty of securing good results.

However, until further experimentation has fully adapted the formalin treatment to practical usage, steam sterilization stands as the best means of preventing tobacco root-rot.

PALLIATIVE MEASURES.

The measures now to be cited are purely secondary to the preventive remedies above described, and serve merely to reduce to a greater or less degree the amount of disease.

Soils containing a good proportion of sand should be used for seed beds and heavy clays avoided or lightened by the addition of sand.

Excessive fertilization and watering should be avoided, and if sash are used thorough ventilation should be given.

The making of new beds each year has in some cases obviated the trouble, though some instances have been noted where the disease has been bad in perfectly fresh beds.

The use of fresh soil in old beds is a doubtful expedient, as spores are inevitably left on the sides of the beds and in the soil beneath, and these may readily infect the new soil and render the work futile. Several instances have come to the writer's attention in which this has been done, with the result that the disease has been as bad as or worse than during the preceding year.

Special care should be taken to avoid too heavy seeding of the beds and subsequent crowding of the plants.

Several Italian writers, Peglion, Benincasa, and Campbell, report differences in resistance to root-rot in the varieties of tobacco in cultivation. At both the Pontecorvo and the San Sepolcro tobacco agencies the varieties Brasile Beneventano and Seedleaf showed the greatest resistance to root-rot, while the Kentucky and the Burley were especially susceptible. Moreover, Benincasa states that "it is an established fact that the indigenous varieties offer a resistance to root-rot much greater than that of exotic varieties, the seed of which is imported every year from their native country."

So far as known, no observations have been made along this line in this country.

SUMMARY.

Root-rot of tobacco is a disease causing the death or dwarfing of the plants in the seed bed and in the field. The trouble has been especially serious for the last few years in the tobacco-growing sections of Connecticut, where it has caused considerable loss.

The disease is due to the attacks of the fungus *Thielavia basicola* upon the roots of the plants.

The fungus belongs to the family Perisporiaceæ and produces three kinds of spores, by means of which it is disseminated. It has been found in seven countries parasitic on twenty-five different host plants.

Thielavia has been grown in artificial culture on a wide range of culture media and its life history studied.

The conditions conducive to serious injury from root-rot are: (1) Infection of the seed bed or the field with *Thielavia basicola;* (2) a fairly heavy soil rich in humus; (3) excessive fertilization; (4) heavy watering in the beds; (5) lack of ventilation in the beds.

The sterilization of infected seed beds by the methods herein described furnishes an effective means of preventing the disease.

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20. Clinton, G. P. Root-rot of tobacco. Thielavia basicola (B. and Br.) Zopf. Conn. Agr. Expt. Sta. 30th Ann. Rpt., 1906, Rpt. Botanist, pp. 342–368. New Haven, 1907.

A complete report of Clinton's investigations of root-rot in Connecticut in 1906, containing a description of the fungus, a discussion of the disease in its various aspects, and reports of experiments, with recommendations for the prevention of the disease in seed beds.

20a. ——— Root-rot of tobacco—II. Conn. Agr. Expt. Sta. 31st Ann. Rpt., 1907, Rpt. Botanist, pp. 363–368. New Haven, 1908.

A further report on cultural work and seed-bed treatments with steam and formalin, from which Clinton concludes that fall applications of formalin, 1 to 100, furnish a very efficient and convenient method of preventing root-rot and that steam treatment is fully as effective but more cumbersome and expensive. The steam-rake method was used.

21. Fischer, Ed. Thielavia, Zopf. Engler, A., and Prantl, K. Die natürlichen Pflanzenfamilien, teil I, abt. 1, p. 299. Leipzig, 1897.

Fischer describes Thielavia as a genus of the Aspergillaceæ in the Plectascineæ. Figures all spore forms, a perithecium, and an ascus.

22. Frank, A. B. Die Krankheiten der Pflanzen. [Aufl. 1.] Breslau, 1880.

See p. 586 for brief description of the fungus *Thielavia basicola.*

23. ——— [Idem, aufl. 2.] Breslau, 1895–1896.

See bd. 2, p. 278, for a fuller description of the fungus.

24. Gafaro. [Reports plants of Kentucky tobacco attacked by root-rot at Sassari, Sardinia.] Bol. Tecnic. d. Coltiv. d. Tabacchi, an. 6, no. 3, p. 203. Scafati, 1907.

25. Galloway, B. T. Commercial violet culture. [Ed. 1.] New York, 1899.

See pp. 170–174, fig. 51. Galloway describes a stem-rot of violets due to Thielavia and gives a method of holding it in check.

26. ——— [Idem, ed. 2.] New York, 1903.

See pp. 174–178, fig. 53.

27. Jaczewski, A. de. Essai de classification naturelle des Pyrenomycetes. Bul. Soc. Mycol. France, t. 10, pp. 13–48. Paris, 1894.

A short description of Thielavia as a genus of the tribe Thielaviées, family Sphaerelées, of the Pyrenomycetes, is given.

28. Kirchner, O. Die Krankheiten und Beschädigungen unserer landwirtschaftlichen Kulturpflanzen. [Aufl. 2.] Stuttgart, 1906.

See pp. 126, 138, 232, 336. Kirchner describes Thielavia as occurring on various hosts, particularly tobacco.

29. Marchal, É. Rapport sur les maladies cryptogamiques étudiées au Laboratoire de botanique de l'Institut agricole de Gembloux, année 1900. Bruxelles, 1901. (Abstract. Ztschr. Pflanzenkrank., bd. 12, no. 4, p. 239. Stuttgart, 1902.)

See pp. 9–10. Notes Thielavia causing death of peas grown in Sach's solution, and its occurrence in other legumes and composites.

30. ——— In Belgien im Jahre 1901 beobachtete pilzparasitäre Krankheiten. Ztschr. Pflanzenkrank., bd. 12, no. 1/2, p. 48. Stuttgart, 1902.

31. Orton, W. A. Plant diseases in 1901. U. S. Dept. Agr., Yearbook, 1901, p. 672. Washington, 1902.

Notes occurrence of Thielavia in seed beds.

32. ——— Plant diseases in 1903. U. S. Dept. Agr., Yearbook, 1903, p. 554. Washington, 1904.

Reported occurrence of Thielavia on tobacco in Ohio, making necessary selection of new lands for seed beds.

33. Peglion, V. Marciume radicale delle piantine di tabacco causato della Thielavia basicola Zopf. Atti R. Accad. Lincei, an. 294, s. 5, Rend. Cl. Sci. Fis., vol. 6, semestre 2, fasc. 2, pp. 52–56. Roma, 1897.

34. ——— [Idem.] Centbl. Bakt., abt. 2, bd. 3, no. 21/22, pp. 580–584. Jena, 1897.

Peglion gives a complete description of the disease and of Thielavia. Attributes virulence of root-rot to poorly prepared seed beds and to too heavy fertilization and watering.

35. ——— Le malattie crittogamiche delle piante coltivate. [Ed. 1.] Casale Monferrato, 1899.

See pp. 185–188. Marciume radicale delle piantine nei semenzai.

36. ——— [Idem, ed. 2.] Casale Monferrato, 1905.

See pp. 203–206. A description of the disease, with recommendations for treatment.

37. Peglion, V. La moria delle piantine nei semenzai. Ricerche intorno ai mezzi de defesa. Staz. Sper. Agr. Ital., vol. 33, fasc. 3, pp. 221–237. Modena, 1900.

Reports having obtained ascospores of Thielavia in pure culture on 3-year-old potato slants revived by the addition of 0.6 per cent tartaric acid. Advises the burning of seed beds to prevent root-rot.

38. Rostrup, E. Plantepatologi. Kjöbenhavn, 1902.

See p. 437. Brief description of root-rot of various legumes due to *Thielavia basicola*, taken from Zopf.

39. Saccardo, P. A. Thielavia basicola, Zopf. Sylloge fungorum, vol. 1, p. 39. Patavii, 1882.

A brief description taken from Zopf. Notes *Torula basicola* Berk. as one of the spore forms of Thielavia.

40. ——— Torula basicola. Sylloge fungorum, vol. 4, p. 257. Patavii, 1886.

Description taken from Berkeley's account.

41. ——— Clasterosporium fragile (Sorok.) Sacc. Sylloge fungorum, vol. 4, p. 386. Patavii, 1886.

Describes Sorokin's *Helminthosporium fragile* here, not recognizing its identity with the chlamydospore stage of *Thielavia basicola*.

42. Selby, A. D. Investigations of plant diseases in forcing house and garden. Ohio Agr. Expt. Sta. Bul. 73. Wooster, Ohio, 1896. (Abstract in Ztschr. Pflanzenkrank., bd. 8, no. 2, p. 91. Stuttgart, 1898.)

See p. 228. Selby found *Thielavia basicola* on the roots of *Begonia rubra* attacked by nematodes.

43. ——— Tobacco diseases and tobacco breeding. Ohio Agr. Expt. Sta. Bul. 156. Wooster, Ohio, 1904.

Root-rot (black-root). See pp. 95–97, figs. 1 and 2. This paper reports having received specimens of Thielavia-diseased tobacco seedlings from southern Ohio in 1899, and again in 1903 from southwestern Ohio. Advises making beds on new soil each year.

44. ——— Soil treatment of tobacco plant beds. Ohio Agr. Expt. Sta. Circ. 59. Wooster, Ohio, 1906.

Fall applications of formalin 1 to 200, 1 gallon per square foot, are advised to prevent black-root (Thielavia), based on successful experiments with the bed-rot (Rhizoctonia).

45. Shamel, A. D. Another blight strikes tobacco. Hartford Daily Courant, May 28, 1906.

Shamel here reports a serious outbreak of root-rot in the tobacco seed beds of several Connecticut towns. Advises sprinkling beds with 1 to 2,000 formalin solution once a week.

46. Smith, Erwin F. Wilt disease of the cotton, watermelon, and cowpea (Neocosmospora nov. gen.). Div. Veg. Phys. and Path., U. S. Dept. Agr., Bul. 17. Washington, 1899.

See pp. 35, 38. Smith mentions the occurrence of *Thielavia basicola* on cowpea and cotton seedlings in experimental pots causing the rotting of stems at or beneath the surface of the soil.

47. Sorauer, P. Handbuch der Pflanzenkrankheiten. [Aufl. 2.] Berlin. 1886.

See bd. 2, pp. 332, 333. Short note on fungus. Recognizes identity of chlamydospores of Thielavia with *Torula basicola* of Berkeley and Broome and *Helminthosporium fragile* of Sorokin.

48. SORAUER, P. Ueber die Wurzelbräune der Cyclamen. Ztschr. Pflanzenkrank., bd. 5, no. 1, pp. 18–20. Stuttgart, 1895.

Details of a root disease of cyclamens caused by *Thielavia basicola* are presented.

49. ——— [Note of occurrence of Thielavia.] Ztschr. Pflanzenkrank., bd. 6, hft. 3, p. 315. Stuttgart, 1896.

50. SOROKIN, N. Ueber Helminthosporium fragile sp. n. Hedwigia, bd. 15, no. 8, pp. 113–114, fig. A. Dresden, 1876.

Chlamydospores of Thielavia found on rotten horse-radish roots at Kazan, Russia, are described as a new species.

51. THAXTER, R. Fungus in violet roots. Conn. Agr. Exp. Sta., Ann. Rpt., 1891, pp. 166–167. New Haven, Conn., 1892.

The occurrence of Thielavia on violets in Connecticut is noted; this is the first report of the presence of the fungus in America.

52. TUBEUF, K. von, and SMITH, W. G. Diseases of plants induced by cryptogamic parasites. New York, 1897.

See pp. 182–183. A short account of the fungus is given, and it is stated that it is the only species of the Perisporieæ which causes a really serious plant disease.

53. VERDURA, O. [Bad outbreak of root-rot in tobacco nurseries at Cori, Italy.] Bol. Tecnic. d. Coltiv. d. Tabacchi, an. 1, no. 3, p. 158. Scafati, 1902.

54. WINTER, G. Thielavia. Rabenhorst. Kryptogamen-flora von Deutschland. Aufl. 2, bd. 1, abt. 2, p. 53. Leipzig, 1887.

This paper is a scientific description of the fungus.

55. WUIOVICH, M. Sulla costituzione e sul trattamento dei terricci da adoperare nei semenzai. Bol. Tecnic. d. Coltiv. d. Tabacchi, an. 6, no. 3, pp. 162–166, 2 pls. Scafati, 1907.

A discussion of experiments with various soils for tobacco seed beds, burned and unburned.

56. ZOPF, W. Thielavia, genus novum, Perisporiacearum. Verhandl. Bot. Ver. Prov. Brandenburg, j. 18, Sitzungsber. 30 Juni, 1876, pp. 101–105. Berlin, 1876.

57. ——— [Idem.] Hedwegia, bd. 16, pp. 114–117. Dresden, 1877.

Zopf describes the fungus found on roots of *Senecio elegans* as a new genus of Perisporieæ, giving it the name Thielavia after Doctor Thielau and placing Berkeley and Broome's *Torula basicola* under it. Mentions four spore forms.

58. ——— Die Pilze. Breslau, 1890.

See pp. 36, 81, 96, and 113, fig. 61. Notes on morphology of Thielavia.

59. ——— Ueber die Wurzelbraüne der Lupinen, eine neue Pilzkrankheit. Ztschr. Pflanzenkrank., bd. 1, no. 2, pp. 72–76, figs. 1 and 2. Stuttgart, 1891.

Describes a brown-root of lupines as a new disease due to Thielavia and gives a description of the fungus and a list of its hosts.

PLATES.

DESCRIPTION OF PLATES.

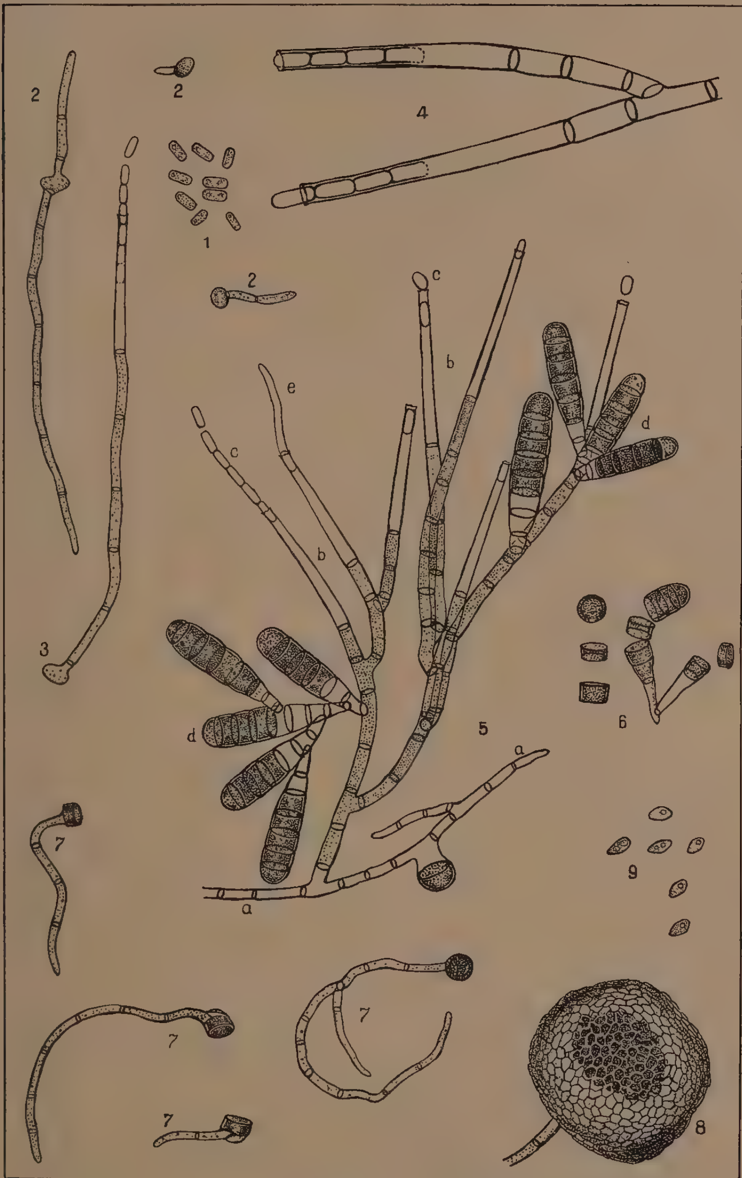
PLATE I. Microscopic characters of the root-rot fungus (*Thielavia basicola* Zopf). 1.—Endoconidia. 2.—Different stages in the germination of the endoconidia. 3.—Endoconidia germinated and producing new endoconidia. 4.—Endoconidiophore, enlarged, showing formation and ejection of endoconidia. 5.—(A) Mycelium. (B) Endoconidial cell. (C) Endoconidia being pushed out. (D) Chlamydospores. (E) Endoconidium germinating in situ. 6.—Chlamydospores breaking up into segments. 7.—Chlamydospore segments germinating. 8.—Perithecium. 9.—Ascospores.

PLATE II. Demonstration of parasitism of Thielavia. Both dishes shown were fitted with moist blotting paper and sterilized in an autoclave. Tobacco seed was then sown, and four days later, when the seed was germinating, dish A was inoculated with a pure culture of Thielavia. Dish B was left uninoculated. The photograph here reproduced was taken two weeks after inoculation.

PLATE III. Fig. 1.—Root-rot on a small tobacco plant grown in sterilized soil and inoculated at the age of two months with a pure culture of Thielavia. Many small rootlets are blackened and dead. Fig. 2.—Badly diseased root system of a tobacco plant from a field at Suffield, Conn., showing all the roots rotted off close up to the stem. Fig. 3.—(A) Root systems of tobacco seedlings grown in sterilized soil inoculated with pure culture of Thielavia and subsequently treated with a formalin solution 1 to 200. (B) Root systems of tobacco seedlings in the same inoculated soil untreated with formalin, badly diseased with root-rot.

PLATE IV. Fig. 1.—Tobacco bed at Simsbury, Conn., badly diseased with Thielavia root-rot in 1906. Plants scattered, stunted, and sickly in appearance. Fig. 2.—Tobacco plants in the same bed in 1907 after it had been steam sterilized for one hour at 175° F. by the inverted-pan method. Note the full stand of vigorous, healthy plants and the absence of weeds. No weeding had been done in this bed. Fig. 3.—(A) Tobacco seedlings grown on soil from a badly diseased bed at Simsbury, Conn. The root systems are all diseased with root-rot. (B) Seedlings on the same soil which had been steam sterilized for one and one-half hours at 120° C. The root systems of all plants are perfectly healthy. Pots A and B were sown on the same day.

PLATE V. Fig. 1.—A spot in a tobacco field at Simsbury, Conn., badly diseased with Thielavia root-rot. Note the stunted condition of the plants in the foreground as compared with those on the left. The root shown in Plate III, figure 2, was from such a spot as this. Fig. 2.—A tobacco seed bed being sterilized with steam by the inverted-pan method. The steam is conducted from the boiler on the right, underneath the pan, by a hose. Note the soil thermometer at ×, inserted 6 or 8 inches below the surface of the soil.



W. W. GILBERT AND J F BREWER

MICROSCOPIC CHARACTERS OF THE ROOT-ROT FUNGUS IN VARIOUS STAGES.

TWO DISHES CONTAINING GROWING TOBACCO SEEDLINGS, AFFORDING A DEMONSTRATION OF THE PARASITISM OF THIELAVIA.

A, Inoculated; *B*, not inoculated.

TOBACCO PLANTS AFFECTED WITH ROOT-ROT.

Fig. 1.—Plants inoculated at two months. Fig. 2.—Diseased root from field. Fig. 3.—Seedlings from soil inoculated with Thielavia: *A*, sterilized with formalin (1 to 200), and, *B*, untreated.

FIG. 1.—A TOBACCO BED BADLY DISEASED WITH ROOT-ROT.

FIG. 2.—TOBACCO PLANTS IN THE SAME BED SHOWN IN FIGURE 1 AFTER IT HAD BEEN STERILIZED WITH STEAM.

FIG. 3.—SEEDLINGS GROWN IN DISEASED SOIL.

A, Not sterilized; *B*, steam sterilized.

FIG. 1.—A DISEASED SPOT IN A TOBACCO FIELD.

FIG. 2.—A TOBACCO BED BEING STERILIZED WITH STEAM BY THE INVERTED-PAN METHOD.

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